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ARTICLE

Porcine Fatty Acid Synthase Gene Polymorphisms Are Associated with Meat Quality and Fatty Acid Composition

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Abstract

We assessed the effects of single-nucleotide polymorphisms (SNPs) within the porcine fatty acid synthase (*FASN*) gene regarding meat quality and fatty acid composition in two pig populations: Korean native pigs (KNP) were crossed with Yorkshire (YS) F_2, and KNP were crossed with Landrace (LR) F_2. Direct DNA sequencing using eight KNP and eight YS pigs revealed two SNPs: c.265C>T (silent) in exon 4 and c.6545A>C (Asn→His) in exon 39. The frequency of the two SNPs was analyzed using the polymerase chain reaction-restriction fragment length polymorphism method in seven pig breeds and their association with meat quality traits and fatty acid composition was studied. In the KNP × YS F_2 population, both SNPs were significantly associated with the level of monounsaturated fatty acids, including palmitoleic (C16:1) and oleic acid (C18:1) ($p<0.005$). c.6545A>C was associated with intramuscular fat content in both populations. Our results indicate that variations in c.265C>T and c.6545A>C of the pig *FASN* can be used to select animals with better fatty acid composition and meat quality. Moreover, KNP was a useful breed for identifying genetic factors affecting meat quality and fatty acid composition and for producing high quality pork.

Key words: fatty acid synthase, single nucleotide polymorphisms, meat quality, fatty acid composition, pig

Introduction

Various economically important traits in pigs have been genetically improved. As a result, current commercial pigs have very high growth rates, good feed conversion rates, and lean carcasses. Because consumers prefer high quality pork with good meat quality, there is a global trend toward high quality pork production, intramuscular fat (IMF) and marbling in particular have been considered the most important traits that correspond to meat quality. Now, the fatty acid composition of triglycerides in pork sirloin is becoming an important attribute that is related to human health (Wood *et al*., 2004a; Wood *et al*., 2004b). The fatty acid composition in pork is 60% saturated fatty acids (SFAs) and 40% unsaturated fatty acids (UFAs); the SFA content of pork is lower than that of beef (De *et al*., 2004). SFAs, including lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (C16:0), have very harmful cardiovascular effects, although stearic acid (C18:0) does not. Monounsaturated fatty acids (MUFAs) have low melting points and improve the tenderness and flavor of pork (Melton *et al*., 1982; Smith *et al*., 2006). The MUFAs in pork, particularly oleic acid (C18:1), prevent oxidation and are very positively correlated with IMF, marbling, and meat quality traits (Cannata *et al*., 2010). In addition, palmitoleic acid (C16:1) intake is effective in reducing low-density lipoprotein (LDL) cholesterol and improves pancreatic function leading to increased insulin secretion (Nestel *et al*., 1994).

For the genetic improvement of pigs to produce high-grade pork, domestic animals with excellent meat quality and fatty acid composition traits should be carefully selected. Several studies have investigated candidate genes related to meat quality and fatty acid composition in pigs, and variation in the acetyl-coenzyme A carboxylase-α gene (*ACACA*) is reported to be a genetic marker of IMF and C18:1 content adjustment. In addition, variation in gastric inhibitory polypeptide (GIP) is also reported to be a genetic marker of C16:1 and C18:0 con-

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tent adjustment (Munoz *et al*., 2007; Gallardo *et al*., 2009). Several studies have reported that cattle *FASN* variation is a genetic factor that directly affects fatty acid content in beef, particularly oleic acid (C18:1), which influences flavor (Zhang *et al*., 2008; Bhuiyan *et al*., 2009; Abe *et al*., 2009; Kim *et al*., 2010a).

The *FASN* gene is located on pig chromosome 12 and encodes a complex enzyme that synthesizes fatty acids in adipose and liver tissues (Munoz *et al*., 2003). The pig *FASN* gene is known to be in the QTL region (0-40 cM), which is associated with fatty acid formation in backfat, and it has been reported that variation in the *FASN* gene affects the gadoleic acid (C20:1, a MUFA) content of the fatty acid components in backfat (Munoz *et al*., 2007). In pigs, crossbreeding between different breeds is used for the selection and improvement of heterosis. Global representative pig breeds used for such improvements include Duroc, Landrace, Yorkshire and Berkshire. If these breeds are to be used to improve pork quality, their traits should be well understood. Compared with the Yorkshire breed, which is a Western breed, Korean native pigs (KNP) have smaller body sizes but thicker backfat, better fat deposits, better meat quality, and better marbling. Thus, several studies have been conducted in order to understand their molecular genetic background with the goal of producing high quality pork (Kim *et al*., 2007; Kim *et al*., 2009; Kim *et al*., 2010b; Li *et al*., 2010; Moon *et al*., 2009).

Therefore, the objectives of this study were to identify variations in porcine *FASN*, to determine if the variations are genetic factors that affect meat quality and fatty acid composition, and then to evaluate the traits of different breeds with respect to the *FASN* gene.

Materials and Methods

Animals and phenotypic traits analyzed

DNA samples from Korean native pigs (n = 48), Landrace (n = 24), Yorkshire (n = 24), Duroc (n = 24), and Berkshire (n = 24) pigs were obtained from the National Institute of Animal Science (NIAS) in Korea. A Korean native pig (KNP)×Yorkshire (YS) population was produced at Chungbuk National University in order to identify QTLs and functional genes for economically important phenotypes (Kim *et al*., 2010b; Li *et al*., 2010). The meat quality traits include crude ash (C-ash), crude protein (C-pro), intramuscular fat (IMF), drip loss (DL), water-holding capacity (WHC), moisture, cooking loss (CL), shear force, pH at 24 h (24-h pH), color score, and total cholesterol. These traits were measured according to standard methods (Oh *et al.,* 2008). The fatty acid profiles were measured based on a well-established method (Folch *et al*., 1957; Lepage and Roy, 1986). The composition ratios of the following fatty acids were calculated as described in Choi *et al*. (2008): myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), and linoleic acid (C18:2) (Table 1). NIAS reference families were constructed from a cross between KNP and Landrace (LR) sows. Five randomly selected KNP boars were mated with 10 LR sows to produce the F_1 animals. An F_1 sire was randomly selected from each litter and mated with full-sib sows. Thus, 10 sires and 31 dams were used to produce 543 F_2 animals (Kim *et al*., 2007). All phenotypic data are summarized at Table 1.

Primer design and PCR

The sequence of exon 4 in the *FASN* gene was analyzed using a previously described primer (Munoz *et al*., 2003). Primers for exons 38-41 of *FASN* were created in two steps. First, GenBank sequences from pig (AY954688), cattle (AF285607), human (AY451392), and goat (DQ915966) were obtained using the NCBI BLAST program (http://blast.ncbi.nlm.nih.gov/), and primers in exon regions 38-41 spanning pig *FASN* introns were designed using Oligo 6 software. These primer sets were then used to sequence the PCR product. Second, primers to amplify exonic regions were also designed within intronic sequences (introns 38-41) (GenBank accesion number HQ651152). The oligonucleotide sequences, annealing temperatures, and PCR product sizes are presented in Table 2.

PCR amplifications were performed in a PTC-200 thermocycler (MJ Research, USA) using a standard protocol. The results of the PCR reaction were verified using 1.5% agarose gel electrophoresis.

Sequencing, polymorphism identification, and genotyping

The DNA samples used in the sequence analysis were obtained from 16 pigs (8 KNP and 8 YS). A total of eight PCR products were sequenced with both forward and reverse primers. Sequencher software (Gene Codes, version 4.6, USA) was used to assemble the sequences and identify DNA polymorphisms. Polymorphic sites were analyzed for putative RFLPs using NEBcutter (http://tools.neb.com/NEBcutter2/index.php). Genotyping of the putative RFLPs was performed on individual DNA samples from five different pig breeds: Duroc, LR, YS, Berkshire, and KNP. All restriction enzymes were supplied by New England BioLabs (Ipswich, USA), and restriction

Table 1. Means and standard deviation of traits measured in pork from Korean native pig × Yorkshire (KY) cross F2 and Korean native pig × Landrace (KL) cross F2

Population	KNP × YS cross F2			KNP × LR cross F2		
	Mean	SD	N	Mean	SD	N
Chemical composition (%)						
Moisture	73.96	1.71	349	-	-	-
Crude Protein (C-pro)	22.18	1.59	349	22.24	1.08	553
Intramuscular fat (IMF)	2.49	1.45	349	2.15	2.15	553
Crude Ash (C-ash)	1.05	0.13	349	1.04	0.14	553
Meat quality characteristics						
Water holding capacity (WHC) (%)	58.03	6.34	349	58.14	5.18	553
24-h pH	5.63	0.25	349	5.63	0.22	553
Drip loss (DL) (%)	5.11	1.81	349	–	–	–
Cooking loss (CL) (%)	32.26	3.53	349	–	–	–
Shear force (kg)	1.73	0.43	349	–	–	–
Cholesterol (mg/100g)	142.02	84.38	349	–	–	–
Subjective evaluation*						
Color (score)	3.06	0.49	349	–	–	–
Texture (score)	2.86	0.42	349	–	–	–
Myristic acid (C14:0) (%)	1.88	1.32	237	–	–	–
Palmitic acid (C16:0) (%)	21.11	2.61	237	–	–	–
Palmitoleic acid (C16:1) (%)	3.91	1.66	237	–	–	–
Stearic acid (C18:0) (%)	11.10	3.38	237	–	–	–
Oleic acid (C18:1) (%)	31.97	5.40	237	–	–	–
Linoleic acid (C18:2) (%)	12.90	6.40	237	–	–	–
Fatty acid composition						

Texture, 1: extremely bad texture, 5: very good texture
Meat color, 1: very pale, 5: very dark
5: very tender, very juicy, very intense

digestions were performed according to the manufacturer's recommendations. Digested PCR products were analyzed on 2.5-4% agarose gels, and each allele was scored manually. The restriction enzymes and polymorphic fragment sizes used for SNP genotyping are given in Table 2.

Statistical analysis

The genotypes of the *FASN* gene (c.265C>T and c.6545A>C) obtained from the testing materials were classified and their frequencies were calculated. In order to examine the genetic equilibrium between the populations (Hardy-Weinberg equilibrium, $P < 0.05$), the significance was tested using the χ^2-test.

Genotypic effects of variations in the *FASN* gene (c.265C >T and c.6545A>C) were estimated with mixed-model analysis using the SAS 9.1 Package/PC and the differences between genotypes were examined using least significant difference tests. The models used in the statistical analysis are as follows:

i) KNP × YS cross F_2 (n = 343)

$$Y_{ijklmnop} = \mu + S_i + G_j + D_k + b_1L_l + B_m + D_n + e_{ijklmno},$$

where $Y_{ijklmnop}$ is the observed value of carcass traits, μ is the mean of the samples, S_i is the effect of sex, G_j is the effect of genotype, D_k is the effect of slaughter date, L_l is the covariate of live weight, b_1 is the regression coefficient of live weight, B_m is the covariate of body length, D_n is the random effect of age at slaughter and $e_{ijklmno}$ is the random error.

ii) KNP × LR cross F_2 (n = 480)

$$Y_{ijklmno} = \mu + S_i + G_j + D_k + b_1L_l + D_m + e_{ijklmn},$$

where Y_{ijklmo} is the observed value of carcass traits, μ is the mean of the samples, S_i is the effect of sex, G_j is the effect of genotype, D_k is the effect of slaughter date, L_l is the covariate of live weight, b_1 is the regression coefficient of live weight, D_m is the random effect of age at slaughter and e_{ijklmn} is the random error. Body length was excluded from the model due to lack of phenotypic data

Table 2. PCR primers and conditions used for amplification and sequencing, and restriction enzymes used for SNP genotyping

Oligonucleotide	GenBank no	Primer Forward(5'→3') Reverse(5'→3')	Annealing temp.	Product size	SNP detection	Restriction enzyme	Size (bp) of the allelic polymorphism
FASN-exon4*	AY183428	ATCAACCCTGCTTCCCTTCGTG CGCGCTGGCAGCCTATCAT	58	130	c.265C>T (Silent)	*Fnu4H* I	120, 66
FASN-intron38	AF285607	GGGCGTCGTCCTGGAGACCAT GCTGCCGCACCTCCG	60	527			
FASN-intron39	AF285607	GGGCCTGGACTCGCTCAT ACGGTGATGGAGCCCTCGATG	57	312	c.6545A>C (Asn->His)	BssS I	312, 161
FASN-intron40	AF285607	GGGGCCTTACCGCATCGC GCAGCCGGGGGTCATCTTAGC	57	265			
FASN-intron41	AF285607	TGCAGCAGTTCACCGACATGG TTGCCGTCGCACACCT	58	295			
FASN-exon39	AF285607	CGGCGACTCCCACATCC GAAGGTGTGTGAGCCGTCGAA	58	507			
FASN-exon40	AF285607	CCCGAGGGGCCTTACCGCATC GAAGGAGCGAGCGGCGAA	60	556			
FASN-exon41	AF285607	CCCGAGGGGCCTTACCGCATC GAAGGAGCGAGCGGCGAA	60	556			

* Primer is referenced from Munos *et al*., 2003.
All primers were based on the porcine *FASN* sequence, NCBI GenBank accession number AF285607.

on this trait for KNP × LR cross F_2.

iii) Combined (KNP × YS) + (KNP × LR) population (n = 822)

$$Y_{ijklmno} = \mu + S_i + G_j + D_k + b_l L_l + D_m + e_{ijklmn},$$

where $Y_{ijklmno}$ is the observed value of carcass traits, μ is the mean of the samples, S_i is the effect of sex, G_j is the effect of genotype, D_k is the effect of slaughter date, L_l is the covariate of live weight, b_l is the regression coefficient of live weight, D_m is the random effect of age at slaughter and e_{ijklmn} is the random error. The least-square means of the genotypes estimated using these equations were tested using the *t*-test, and the phenotypic differences between haplotypes were tested using contrast tests.

The degree of linkage disequilibrium (LD) between variations in the *FASN* gene, c.265C>T (silent) and c.6545A >C (Asn→His), was estimated using the HaploView software package (Barrett *et al*., 2005), and the D' and r^2 values between the variations were estimated using the method described by Stephens *et al*. (2001).

The association of three haplotype classes with collected economic traits was also analyzed using the same model used to analyze the effects of individual genotypes.

Results

SNP detection and genotyping

The pig *FASN* sequence, including exon 4 and exons 38-41 sequence (GenBank accession number HQ651152), spanning four introns, was obtained using eight primer sets. Two SNPs were found, both of which have been previously reported: c.265C>T (silent) in exon 4 and c.6545A >C (Asn→His) in exon 39 (Munoz *et al*., 2007). These polymorphisms in the pig *FASN* gene were analyzed using PCR-RFLP methods; c.265C>T with *Fnu4*HI and c.6545A>C with *Bss*SI. For the c.265C>T polymorphism, restriction digestion yielded fragments of 120 and 10 bp when the variation was the CC genotype; 120, 66, 54, and 10 bp for the CT genotype; and 66, 64, and 10 bp for the TT genotype. For the c.6545A>C polymorphism, restriction digestion yielded a fragment of 312 bp when the variation was the AA genotype; 312, 161, and 151 bp for the AC genotype; and 161 and 151 bp for the CC genotype (Table 2).

FASN amino acid alignment among mammalian species

The putative porcine *FASN* amino acid sequence encoded by exons 39-42 (AAX55638) was compared to that of human, goat, and cattle (Fig. 1). One of the amino acid positions mutated in the porcine sequence, His2206Asn is

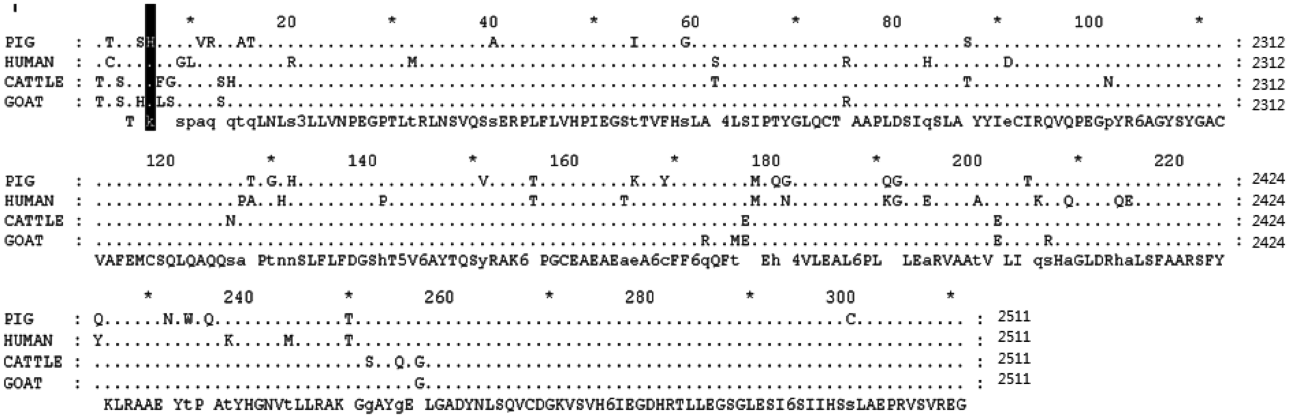

Fig. 1. The putative bovine *FASN* amino acid sequences encoded by exons 39-42 (AAX55638) compared with those in humans, goats, and cattle. Sequences were aligned using Blast (http://www.ncbi.nlm.nih.gov/blast/Blast). One of the mutated amino acid positions, 2206 (H or N), is represented by a black bar.

Table 3. Genotypes and minor allele frequencies of 2 (c.265C>T and c.6545A>C) polymorphisms in the *FASN* gene from 7 pig populations

SNP Position	AA change	Population (N. of pig)	Genotype (N. of pig)			Allele 1 frequency	Allele 2 frequency	Heterozy-gosity	HWE P-value
c.265C>T	Silent	Landrace (n=24)	CC (6)	CT (15)	TT (3)	0.44	0.56	0.492	0.4183
		Yorkshire (n=24)	CC (5)	CT (14)	TT (5)	0.50	0.50	0.500	0.7595
		Duroc (n=24)	CC (24)	CT (0)	TT (0)	1	0	0	1.0000
		Berkshire (n=24)	CC (17)	CT (7)	TT (0)	0.85	0.15	0.249	0.0912
		KNP (n=48)	CC (47)	CT (1)	TT (0)	0.99	0.01	0.021	1.0000
		KY (n=342)	CC (159)	CT (163)	TT (20)	0.70	0.30	0.204	0.5679
		KL (n=343)	CC (270)	AG(67)	TT (6)	0.90	0.10	0.417	0.0118
c.6545A>C	Asn->His	Yorkshire (n=21)	AA (3)	CA (11)	CC (7)	0.40	0.60	0.482	1.0000
		Landrace (n=20)	AA (4)	CA (12)	CC (4)	0.50	0.50	0.500	0.7374
		Duroc (n=24)	AA (5)	CA (14)	CC (5)	0.50	0.50	0.500	0.7595
		Berkshire (n=22)	AA (10)	CA (11)	CC (1)	0.73	0.27	0.416	0.7692
		KNP (n=48)	AA (48)	CA (0)	CC (0)	1	0	0	1.0000
		KY (n=326)	AA (127)	CA (172)	CC (27)	0.65	0.35	0.408	0.0571
		KL (n=480)	AA (264)	CA (174)	CC (42)	0.73	0.27	0.453	0.0641

KY: Korean Native pig × Yorkshire cross F2 pigs
KL: Korean Native pig × Landrace cross F2 pigs

shown in a black box in Fig. 1; in all the other species, amino acid position 2206 was fixed to lysine (K) (Fig. 1).

Comparison of FASN genotype frequencies among several pig breeds

The genotype frequencies of c.265C>T (exon 4 - silent) and c.6545A>C (exon 39 - Asn→His) variation in the *FASN* gene from different pig breeds are summarized in Table 4. The frequency of the C allele in c.265C>T was 0.44 and 0.50 in the Landrace and Yorkshire populations, respectively. However, the C allele frequency was 1, 0.85, and 0.99 in the Duroc, Berkshire and Korean native pig populations, respectively. For the second polymorphism, c.6545A>C, located in exon 39, the A allele frequency was 0.40, 0.50 and 0.50 in the Landrace, Yorkshire, and Duroc populations, respectively. However, the A allele frequency was 0.73 and 1 in the Berkshire and Korean native pig populations, respectively (Table 3).

Single polymorphism linkage analyses

The association of the two polymorphisms in the pig *FASN* gene, c.265C>T (exon 4 - silent) and c.6545A>C (exon 39 - Asn→His), with traits related to meat quality and fatty acid composition was analyzed using (1) the KNP × YS F_2 population (n = 347), and (2) the KNP × LR F_2 population (n = 553) (Table 4). There was a significant association between the c.265C>T genotype located in exon 4 and both C-pro and flavor in the KNP × YS F_2

Table 4. Association of 2 *FASN* gene polymorphism (c.265C>T and c.6545A>C) genotypes with five meat quality traits from KNP × YS and KNP × LR cross F2 pigs

Gene	Phenotypic trait	KNP x YS cross F2 (n=342 and 326)			P-value	KNP x LR cross F2 (n=343 and 480)			P-value	Combined KY and KL population (n=685 and 806)			P-value
		Genotypic least squares means (SE)				Genotypic least squares means (SE)				Genotypic least squares means (SE)			
		11	12	22		11	12	22		11	12	22	
FASN Exon4 c.265C>T	C-pro	CC 22.043 (0.116)[a]	CT 22.440 (0.116)[a]	TT 21.892 (0.304)[b]	0.0291	CC 22.156 (0.148	CT 22.205 (0.205)	TT 21.775 (0.512	0.7455	CC 22.196 (0.101)	CT 22.205 (0.138)	TT 22.060 (0.475)	0.9610
	IMF	CC 2.335 (0.131)	CT 2.730 (0.132)	TT 2.875 (0.348)	0.0686	CC 2.250 (0.183)	CT 2.131 (0.250)	TT 2.703 (0.236)	0.0701	CC 2.409 (0.120)[a]	CT 2.258 (0.164)[a]	TT 2.749 (0.564)[b]	0.6393
	C-Ash	CC 1.068 (0.011)	CT 1.066 (0.011)	TT 0.988 (0.029)	0.0352	CC 1.016 (0.018	CT 1.056 (0.026	TT 0.996 (0.065	0.1924	CC 1.040 (0.011)	CT 1.074 (0.015)	TT 1.031 (0.052)	0.2537
	WHC	CC 58.586 (0.369)	CT 58.066 (0.371)	TT 56.511 (0.966)	0.1200	CC 59.140 (0.671[a]	CT 57.128 (0.930)[a, b]	TT 55.144 (2.318)[b]	0.0150	CC 58.980 (0.389)[a]	CT 57.243 (0.531)[a, b]	TT 55.390 (1.825)[b]	0.0164
	24-h pH	CC 5.635 (0.010)	CT 5.636 (0.010)	TT 5.598 (0.026)	0.3807	CC 5.625 (0.029)	CT 5.552 (0.041)	TT 5.534 (0.103)	0.1005	CC 5.654 (0.014)	CT 5.587 (0.019)	TT 5.553 (0.068)	0.0237
FASN Exon39 c.6545A>C	C-pro	AA 22.137 (0.126)	CA 22.341 (0.112)	CC 21.906 (0.261)	0.2216	AA 22.259 (0.158)	CA 21.935 (0.173)	CC 22.202 (0.252)	0.0926	AA 22.335 (0.105)[a]	CA 21.970 (0.107)[b]	CC 22.276 (0.221)[b]	0.0271
	IMF	AA 2.731 (0.295)[a]	CA 2.712 (0.126)[a]	CC 2.324 (0.141)[b]	0.0390	AA 2.260 (0.199)[a]	CA 2.311 (0.219)[a]	CC 1.956 (0.317)[b]	0.0107	AA 2.384 (0.126)[a]	CA 2.393 (0.129)[a]	CC 2.273 (0.265)[b]	0.0487
	C-Ash	AA 1.067 (0.011)[a]	CA 1.069 (0.010)[a]	CC 1.003 (0.024)[b]	0.0448	AA 1.020 (0.020)	CA 1.027 (0.022)	CC 1.018 (0.032)	0.9929	AA 1.050 (0.011)	CA 1.057 (0.011)	CC 1.035 (0.024)	0.7217
	WHC	AA 58.723 (0.409)	CA 58.042 (0.365)	CC 57.400 (0.853)	0.2653	AA 59.422 (0.723)[a]	CA 58.674 (0.791)[a, b]	CC 56.230 (1.153)[b]	0.0192	AA 58.833 (0.408)[a]	CA 57.859 (0.416)[a, b]	CC 56.541 (0.858)[b]	0.0406
	24-h pH	AA 5.638 (0.010)	CA 5.637 (0.009)	CC 5.593 (0.022)	0.1751	AA 5.632 (0.032)	CA 5.608 (0.0395)	CC 5.538 (0.051)	0.1692	AA 5.647 (0.015)[a]	CA 5.619 (0.015)[a]	CC 5.555 (0.031)[b]	0.0355

Significance level: [a, b] 0.05; [c, d] 0.01; [e, f] 0.005

Table 5. Association of 2 *FASN* gene polymorphism (c.265C>T and c.6545A>C) genotypes with meat quality and fatty acid composition traits from KNP × YS cross F2 pigs

Gene	Phenotypic trait	KNP x YS cross F2 (n=342 and 326)			P-value
		Genotypic least squares means (SE)			
		11	12	22	
FASN Exon4 c.265C>T	Shearforce	CC: 1758.95 (36.704)	CT: 1767.28 (36.904)	TT: 1531.91 (97.253)	0.0701
	Flavor	CC: 2.883 (0.033)[a]	CT 2.994 (0.034)[b]	TT: 2.849 (0.089)[b]	0.0437
	C14:0	CC: 2.008 (0.133)	CT 1.721 (0.142)	TT: 1.725 (0.343)	0.3273
	C16:0	CC: 21.175 (0.257)	CT 21.017 (0.274)	TT: 19.595 (0.664)	0.0884
	C16:1	CC: 5.544 (0.224)[e]	CT 3.704 (0.175)[f]	TT: 3.725 (0.154)[f]	0.0002
	C18:0	CC: 10.789 (0.359)	CT 11.517 (0.383)	TT: 9.995 (0.927)	0.1855
	C18:1	CC: 32.828 (0.845)[c]	CT 33.180 (0.515)[c]	TT: 30.891 (0.482)[d]	0.0060
	C18:2	CC: 13.808 (0.356)	CT 12.822 (0.380)	TT: 12.838 (0.920)	0.1606
FASN Exon39 c.6545A>C	Shearforce	AA: 1765.72 (39.513)[a]	CA: 1773.44 (35.075)[a]	CC: 1542.68 (81.794)[b]	0.0309
	Flavor	AA: 2.888 (0.032)[a]	CA: 2.983 (0.028)[b]	CC: 2.854 (0.071)[a, b]	0.1006
	C14:0	AA: 2.082 (0.149)	CA: 1.732 (0.125)	CC: 1.700 (0.322)	0.1812
	C16:0	AA: 21.197 (0.289)	CA: 21.035 (0.243)	CC: 19.728 (0.625)	0.1014
	C16:1	AA: 5.290 (0.402)[e]	CA: 3.654 (0.156)[f]	CC: 3.814 (0.186)[f]	0.0008
	C18:0	AA: 10.727 (0.404)	CA: 11.414 (0.340)	CC: 10.105 (0.873)	0.2218
	C18:1	AA: 32.902 (0.868)[e]	CA: 32.929 (0.455)[e]	CC: 30.562 (0.541)[f]	0.0036
	C18:2	AA: 13.801 (0.402)	CA: 13.032 (0.338)	CC: 12.860 (0.868)	0.3067

Significance level: [a, b] 0.05; [c, d] 0.01; [e, f] 0.005

population (p<0.05), and significant effects on WHC were observed in the KNP × LR F_2 population (p<0.05). It was apparent that animals with the CC genotype had better WHC values than animals with a CT or TT genotype. In addition, we combined the KNP × YS F_2 and KNP × LR F_2 populations to analyze five meat quality measurements in the combined population: C-pro, IMF, C-ash, WHC and 24-h pH, which have common phenotypic data between them. Of the five meat quality measurements in the combined population, WHC and 24-h pH showed significant differences; individuals with the CC genotype had better WHC and higher 24-h pH than individuals with a CT or TT genotype (Table 4). Data from the fatty acid composition analysis of the KNP × YS F_2 population showed highly significant effects on C16:1 and C18:1 (p = 0.0002 and 0.0060, respectively) (Table 5). Animals with a CC or CT genotype had much higher C16:1 and C18:1 (MUFAs) contents than individuals with the TT genotype.

A significant association was also observed between the c.6545A>C genotype in exon 39 and IMF, C-ash and shear force in the KNP × YS F_2 population (p<0.05). IMF, C-ash and shear force were higher in individuals with an AA or CA genotype than in those with the CC genotype. In the KNP × LR F_2 population, the c.6545A >C genotype showed significant linkage with IMF and WHC (p<0.05). Again, animals with an AA or CA genotype had higher IMF and shear force than those with the CC genotype. Of the five meat quality traits in the combined population, C-pro, IMF, WHC, and 24-h pH showed a significant association (p<0.05). Animals with an AA or CA genotype had higher IMF and better WHC than those with the CC genotype (Table 4). The fatty acid composition analysis of the KNP × YS F_2 population had highly significant effects on C16:1 and C18:1 (P = 0.0008 and 0.0036, respectively); individuals with an AA or CA genotype had higher C18:1 contents than individuals with the CC genotype (Table 5).

Discussion

It has been reported that porcine *FASN*, located on chromosome 12, is a candidate gene the fatty acid levels in meats (Munoz *et al*., 2003). It has also been reported that a total of 10 SNPs were detected in the coding region of the pig *FASN* gene; three of these—c.1254A>G (Arg→Gln), c.3189T>C (Thr→Ile) and c.6545A>C (Asn→His)—are missense SNPs that change amino acids, but the phenotypic association has only been established for the c.1254A>G (Arg→Gln) SNP, which adversely affects C20:1 in backfat when it is an A allele (Arg); (Munoz *et al*., 2007). In this study, two SNPs, c.265C>T (Silent) and c.6545A>C (Asn→His), were rediscovered and characterized in several commercially important pig breeds and

their association with fatty acid composition and meat quality was studied. The c.6545A>C (Asn→His) variation in exon 39 was found at amino acid position 2207 in pig FASN; although it is H in pigs, it is fixed as K in cattle, human, and goat (Fig. 1).

FASN is a complex homodimeric enzyme that regulates the *de novo* synthesis of long-chain fatty acids in mammals and catalyzes the formation of 16-carbon fatty acids from acetyl-coenzyme A to malonyl-coenzyme A (Chakravarty *et al*., 2004). This synthesis involves a conserved set of chemical reactions for the cyclic-step elongation of activated precursors by two carbon units (Smith *et al*., 2003). The growing fatty acid is attached to an acyl carrier protein (ACP) throughout its synthesis and is generally released by a thioesterase (TE) when the chain reaches 16 carbons in length. The TE domain has a groove structure close to the catalytic center that can accommodate long fatty acyl chain substrates. The groove is close to the amino terminus of the TE domain, which is linked to the ACP domain of FASN; this holds the growing acyl chains for scanning and release by the TE domain after reaching the optimal fatty acid chain length of 16 carbons (Chakravarty *et al*., 2004). Thus, the spatial organization of the TE domain is crucial for its function as a substrate-binding site in fatty acid synthesis. Maier (2008) reported that the TE domain of the human *FASN* gene is located after amino acid 2199. The c.6545A>C (Asn→His) variation in pig *FASN* exon 39 found in this study is amino acid 2206, which is within the TE domain (Fig. 1).

Morris *et al*. (2007) found five SNPs in the cattle *FASN* gene and reported that they are associated with fatty acid content in adipose tissues and milk lipids. Furthermore, Zhang *et al*. (2008) report that the g.17924A>G variation in the TE domain of the cattle *FASN* gene affects C14:0, C16:0, and C18:1 fatty acid components in beef. There are also some reports indicating that SNPs in the cattle *FASN* TE domain significantly affect backfat thickness and IMF (Abe *et al*., 2009; Li *et al*., 2009; Kim *et al*., 2010a). It has also been reported that when marbling increases, the ratio of C18:1 increases along with changes in fatty acid composition ratios (Smith *et al*., 2006). Moreover, it is known that there is a positive correlation between C18:1 and meat flavor (Cameron and Enser, 1991).

We analyzed the association between: c.265C>T (silent) and c.6545A>C (Asn→His) and meat quality and fatty acid composition traits. Highly significant associations were found between both polymorphisms and C16:1 and C18:1 content, which are MUFAs, in the KNP × YS F_2 population (Table 4). Regarding the effect of c.265C>T (silent) genotype variation, individuals with the CC genotype had relatively higher C16:1 and C18:1 content than individuals with a CT or TT genotype. Regarding the effect of c.6545A>C (Asn→His) genotype variation in the TE domain, individuals with the AA genotype had relatively higher C16:1 and C18:1 content than individuals with a CA or CC genotype. In addition, the linkage analysis between c.6545A>C (Asn→His) variation and meat quality traits revealed particularly interesting results in that significant effects on IMF were common to both the KNP × YS F_2 and KNP × LR F_2 populations. Moreover, individuals with the AA genotype had higher IMF content than individuals with a CA or CC genotype. In addition, when the KNP × YS F_2 and KNP × LR F_2 populations were combined and analyzed, the results revealed significant effects on IMF ($p<0.05$). These results suggest that individuals with the A allele in c.6545A>C (Asn→His) have high IMF, C16:1 and C18:1 content. The frequency of the A allele in the five pig breeds was examined through the analysis of the c.6545A>C (Asn→His) genotypes, and the results show that the frequency of the A allele was higher in the Korean native (1.00) and Berkshire (0.73) breeds than in the Landrace (0.50), Yorkshire (0.40), or Duroc (0.50) breeds. According to a report by Munoz *et al*. (2007), the A allele, which was fixed in KNP, is also fixed in Iberian pigs.

This study revealed that the *FASN* gene is closely linked with variation of fatty acids that affect the meat quality of pigs and that the KNP breed has better meat quality and fatty acid composition than foreign breeds. These results provide an important basis for discovering genetic factors related to diverse and differential meat quality and fatty acid composition in different pig breeds. The results indicate that using KNP in improvement systems would be very beneficial in order to produce high-grade pork.

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